PB84-102375

Toxicity of Acenaphthene and Isophorone to Early Life Stages of Fathead Minnows (Journal Version)

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1982

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1. REPORT NO.	2.	3
EPA-600/J-82-381		PB84-102375

4. TITLE AND SUBTITLE	5. REPORT DATE
Toxicity of Acenaphthene and Isophorone to Early Life Stages of Fathead Minnows (Journal Version)	1982
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Michael A. Cairns and Alan V. Nebeker	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Environmental Research Laboratory U.S. Environmental Protection Agency Corvallis, Oregon 97333	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Environmental Research Laboratory U.S. Environmental Protection Agency Corvallis, Oregon 97333	inhouse
	14. SPONSORING AGENCY CODE
	EPA/600/02

15. SUPPLEMENTARY NOTES

Published in Arch. Environm. Toxicol., 11: 703-707, 1982

16. ABSTRACT

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micrograms/L

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to public	unclassified	5
	20. SECURITY CLASS (This page)	22. PRICE
	unclassified	

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
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EPA Form 2220-1 (9-73)

EPA-600/J-82-381
JOURNAL ARTICLE

Arch. Environm. Contam. Toxicol. 11, 703–707 (1982)

Archives of Environmental Contamination and Toxicology

Toxicity of Acenaphthene and Isophorone to Early Life Stages of Fathead Minnows

Michael A. Cairns and Alan V. Nebeker

U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory, Freshwater Division, 200 S.W. 35th Street, Corvallis, Oregon 97333

Abstract. Flow-through 96-hr and early-life-stage toxicity tests were conducted with acenaphthene and isophorone, using fathead minnows (*Pimephales promelas*) as test animals. The 96-hr LC_{50}'s were 608 μg/L for acenaphthene and 145 and 255 mg/L for isophorone, depending on fish age. No-effect concentrations from early-life-stage exposures were 413 μg acenaphthene/L and 14 mg isophorone/L; these showed good agreement with published toxicity data.

Acenaphthene and isophorone are listed as priority pollutants by the U.S. Environmental Protection Agency (U.S. EPA 1980c), but little research has been conducted on aquatic effects of either chemical. The existing data base is limited to a few static acute toxicity tests with unmeasured concentrations (U.S. EPA 1978b; Le Blanc 1980; and Buccafusco *et al.* 1981). Because of this paucity of data, ambient water quality criteria have not been established for either compound (U.S. EPA 1980a, 1980b).

Acenaphthene (1,2-dehydro-acenaphthylene or 1,8-ethylene-naphthalene) is a white crystalline solid polynuclear aromatic hydrocarbon of low water solubility used in plastics manufacturing, as a dye intermediate, and as a pesticide (U.S. EPA 1980a). It has been found in coal tar from high-temperature coking of coal (Andreikova and Kogan 1977), cigarette smoke (Severson *et al.* 1976), gasoline exhaust (Grimmer *et al.* 1977), and urban air (Cleary 1962). It has been detected in effluents from wood preservative, pesticide, and petrochemical industries (U.S. EPA 1978a), and has been found in well water (Burnham *et al.* 1972), and in a river in Holland (Meijers and Van der Leer 1976).

Isophorone (3,5,5-trimethyl-2-cyclohexen-1-one, trimethyl cyclohexanone, or isoacetophorone) is a clear to yellow liquid of relatively low water solubility, viscosity, and volatility with a pungent camphor- or peppermint-like odor. Prepared commercially from acetone (Rowe and Wolf 1963), it is used mainly as a solvent for vinylic resins applied by roller coating (Blackford 1975), but is also used as a solvent for cellulose derivatives, lacquers, and herbicides. Isophorone is also useful as an intermediate in the manufacture of several alcohols and plant growth retardants (Haruta *et al.* 1974). Annual production for 1973 was estimated to be 14,318,182 kg (U.S. EPA 1980b) and the National Institute for Occupational Safety and Health (NIOSH 1978) has estimated that 1,500,000 workers are exposed annually. In the environment, isophorone has been found in the Delaware River as a general industrial contaminant (Sheldon and Hites 1978) but not in ambient air, soil, or food. Little or no data are available concerning bioconcentration, persistence, or fate. However, its broad application as an industrial solvent suggests that the potential exists for water contamination.

This study was conducted to obtain acute and early-life-stage toxicity values, and to gain insight into threshold toxicity concentrations of acenaphthene and isophorone to fathead minnows (*Pimephales promelas*) in flow-through exposure systems. Embryos and early juveniles were used because these early developmental stages are generally the most sensitive to toxicants (Macek and Sleight 1977; McKim 1977).

0090–4341/82/0011–0703 $01.00
1982 Springer-Verlag New York Inc.

Methods

Early-life-stage flow-through toxicity tests (Stephan 1979) were conducted with fathead minnows to determine concentrations of acenaphthene and isophorone affecting survival and growth and to estimate lower and upper chronic endpoints. A flow-through system was also used to conduct 96-hr acute toxicity tests (Stephan 1975) with both toxicants. A diluter (Garton 1980) was used for toxicant delivery with additional boxes to split the flow to each of two-four replicate test chambers (Tables 1–3) which were partially submerged in heated water (in 38-L aquaria) to control temperature. A freshwater control and five toxicant concentrations with a dilution factor of 0.5 were used in the early-life-stage tests. Test chambers were 1-L glass beakers containing 900 ml of solution, 10.5 cm deep. Water drained from each beaker through a 96-mesh screen secured over a notch cut in the top. Each replicate test chamber contained one embryo screen tray, which was 5.7 cm square and made of 24-mesh stainless steel with a 1-cm lip and suspended 4.8 cm above the bottom of the chamber on a glass tripod. Beakers with 24-mesh screens were substituted on day 17 to facilitate self-cleaning. The flow-rate through each beaker was 55 ± 5 ml/min (88 volume additions in 24 hr). The organisms were always submerged when on the screen trays.

In the first early-life-stage test with acenaphthene, the compound was dissolved in dimethylformamide and injected into the first chamber of the diluter by a syringe pump. A solvent control was employed at the same concentration as in the highest acenaphthene treatment. In the second early-life-stage test with acenaphthene, the compound was dissolved by passing dilution water through two glass columns packed with quartz sand and toxicant crystals; therefore, no solvent control treatment was necessary. In the early-life-stage test with isophorone, 100% isophorone was metered into a premixing box and then into the first chamber of the diluter; no solvent control was necessary.

Dilution water was obtained from wells at the Western Fish Toxicology Station and is chemically described by Samuelson (1976). Dissolved oxygen, pH, total alkalinity, and total hardness were usually determined twice weekly during testing from a control chamber, the highest toxicant treatment, and in an intermediate treatment chamber. Methods were those recommended by the American Public Health Association (APHA *et al.* 1980) and the Environmental Protection Agency (U.S. EPA 1974). Dissolved oxygen averaged 7.9 mg/L (range 6.2 to 8.4); pH averaged 7.4 units (range 7.1 to 7.6); total hardness averaged 35 mg/L as $CaCO_3$ (range 24 to 70); and total alkalinity averaged 28 mg/L as $CaCO_3$ (range 20 to 47) during the toxicity tests. Water temperature was monitored continuously on thermograph charts and averaged 24.5 ± 1°C. A 16-hr light:8-hr dark photoperiod was provided by five 20 W fluorescent tubes, resulting in about 270 lux intensity at the water surface.

Toxicant samples were collected twice weekly and analyzed by high performance liquid chromatography (Waters Associates Model 440) using acetonitrile:water (80:20) as the solvent.

Early-life-stage tests were initiated by placing ≤48-hr-old embryos on screen trays in test chambers. They were obtained from a fathead minnow stock culture maintained in the same water and at the same temperature as during tests. From one to four chambers per treatment were started on the same day depending on egg availability. Eggs were taken from two-four spawning substrates (Benoit and Carlson 1977) on each day an exposure was begun (Gast and Brungs 1973). From 42 to 85 embryos per test chamber were placed on screen trays, depending on the test.

After 40–48 hr of exposure, the dead and unfertilized eggs were counted with a magnifying viewer and discarded. The remaining healthy embryos were randomly reduced to about eight per chamber. Newly-hatched fry could swim into the test chambers from the open-topped screen trays, eliminating handling of young fish. Fish were fed live, newly-hatched brine shrimp at least twice daily on weekdays and once daily on weekends. Fry were fed to satiation at each feeding. Fish were counted thrice weekly and dead fish were removed. Toxicant exposures lasted from 32 to 35 days. At test termination, all fish were measured for fork length to the nearest 0.5 mm and weighed to the nearest mg after blotting.

All early-life-stage data were statistically analyzed with two-way analysis of variance (ANOVA) after arcsin transformation (for percentages) or conversion to logs (for weights and lengths). ANOVA was used to discern differences between control and solvent control treatments, replicates, and treatments. When ANOVA indicated differences among treatments, Williams' (1971) multiple comparison test was conducted to determine which treatments differed significantly ($P = 0.05$) from controls, which allowed us to determine lower and upper chronic endpoints for both chemicals. Geometric means of these chronic limits were calculated and used to predict no-effect concentrations for comparison with published toxicity data.

Acute flow-through toxicity tests (Stephan 1975) were conducted with young fathead minnows of different ages. Three-week and six- to eight-week-old fatheads were used for the isophorone tests, while two-week-old fatheads were used in the acenaphthene experiment. Median lethal (LC_{50}) concentrations and 95% confidence intervals (c.i.) for both compounds were calculated by the trimmed Spearman-Karber method (Hamilton *et al.* 1977, 1978). Test vessels and other physical/chemical parameters except toxicant concentrations were identical to those for the early-life-stage tests. In isophorone acute tests, ten fish per chamber and two chambers per treatment were employed. In the first isophorone test, concentration ranges were between 59 and 394 mg/L; in the second test, between 73 and 412 mg/L. In the acenaphthene acute test, one chamber per treatment, containing ten fish, was used; acenaphthene concentration ranges were between 572 and 1600 μg/L.

Results

Early-life-stage toxicity tests indicated that survival was affected by 682 μg acenaphthene/L and higher, but not by 509 μg/L and less (Table 2), while growth was reduced by 495 μg/L and higher, but not by 345 μg/L and less (Tables 1 and 2). A geometric mean of the lower and upper chronic endpoints, as determined in early-life-stage tests, was 413 μg/L.

An early-life-stage toxicity test indicated that survival was affected by 112 mg isophorone/L but not by 56 mg/L and lower (Table 3). Fork length was affected by 30 mg/L and higher but not by 19 mg/L and lower; weight was affected by 19 mg/L and higher but not by 11 mg/L (Table 3). A geometric mean of the lower and upper chronic endpoints, as determined in early-life-stage tests, was 14 mg/L.

The acute toxicity test with acenaphthene on two-week-old fish resulted in a 96-hr LC_{50} value of 608 μg/L (c.i. = 520–710). The acute toxicity (96-hr

Table 1. Summary of survival and growth effects on fathead minnow early life stages—acenaphthene test 1

Concentration (μg/L) (±SD)	Replicate	No. embryos	No. fish at day 34	Fork length (mm) ($\bar{x}$ ± SD)	Wet weight (mg) ($\bar{x}$ ± SD)	% survival[b]	
Solvent control	1	7	7	16.0	37	100	>88
	2	8	6	(1.4)	(11)	75	
Control	1	8	7	15.5	31	88	>72
	2	9	5	(1.5)	(10)	56	
67	1	8	6	16.5	45	75	>66
(±16)	2	9	5	(2.0)	(17)	56	
107	1	8	7	14.0	30	88	>69
(±24)	2	8	4	(2.8)	(17)	50	
160	1	8	8	14.0	30	100	>72
(±40)	2	9	4	(2.6)	(17)	44	
332	1	7	7	14.5	29	100	>88
(±53)	2	8	6	(1.7)	(10)	75	
495	1	8	4	12.5[a]	17[a]	50	>70
(±171)	2	9	8	(2.1)	(9)	89	

[a] Significantly lower than controls (P = 0.05)
[b] No significant differences

Table 2. Summary of survival and growth effects on fathead minnow early life stages—acenaphthene test 2

Concentration (μg/L) (±SD)	Replicate	No. embryos	No. fish at day 32	Fork length (mm) ($\bar{x}$ ± SD)	Wet weight (mg) ($\bar{x}$ ± SD)	% survival	
Control	1	9	5	13.5	23	56	
	2	9	7	(1.8)	(11)	78	>84
	3	5	5			100	
	4	7	7			100	
197	1	9	9	12.5	18	100	
(±76)	2	8	8	(2.2)	(12)	100	>87
	3	8	7			88	
	4	10	6			60	
345	1	9	8	12.5	18	89	
(±49)	2	8	8	(1.7)	(8)	100	>88
	3	8	6			75	
	4	8	7			88	
509	1	8	5	12.0[a]	16[a]	62	
(±154)	2	7	7	(1.8)	(9)	100	>72
	3	9	7			78	
	4	8	4			50	
682	1	7	2	11.0[a]	11[a]	29	
(±261)	2	10	4	(1.1)	(4)	40	>46[a]
	3	8	2			25	
	4	8	7			88	
1153	1	9	1	8.0[a]	3[a]	11	
(±232)	2	9	0			0	>3[a]
	3	9	0			0	
	4	6	0			0	

[a] Significantly lower than controls (P = 0.05)

Table 3. Summary of survival and growth effects of isophorone on fathead minnow early life stages

Concentration (mg/L) (±SD)	Replicate	No. embryos	No. fish at day 35	Fork length (mm) ($\bar{x}$ ± SD)	Wet weight (mg) ($\bar{x}$ ± SD)	% survival	
Control	1	8	7	14.0	26	88	
	2	8	8	(1.9)	(10)	100	>87
	3	8	6			75	
	4	7	6			86	
11	1	9	7	14.0	24	78	
(±6)	2	8	7	(1.2)	(8)	88	>85
	3	8	6			75	
	4	8	8			100	
19	1	9	8	13.0	18[a]	89	
(±9)	2	8	8	(14)	(6)	100	>88
	3	8	7			88	
	4	12	9			75	
30	1	9	5	12.0[a]	15[a]	56	
(±11)	2	8	7	(1.2)	(5)	88	>83
	3	8	7			88	
	4	8	8			100	
56	1	8	6	11.5[a]	13[a]	75	
±13)	2	8	6	(1.6)	(7)	75	>75
	3	8	6			75	
	4	8	6			75	
112	1	8	0	10.0[a]	6[a]	0	
(±15)	2	8	0	(0.8)	(1)	0	>9[a]
	3	8	1			12	
	4	8	2			25	

[a] Significantly lower than controls (P = 0.05)

LC_{50}) with isophorone and six- to eight-week-old fish was 255 mg/L (c.i. = 229–283), and with three-week-old fish the 96-hr LC_{50} value was 145 mg/L (c.i. = 132–159).

Discussion

The 96-hr LC_{50} value for acenaphthene (608 μg/L) was about 35% of that of 1,700 μg/L determined in static tests with bluegills, *Lepomis macrochirus* (U.S. EPA 1978b; Buccafusco *et al.* 1981). Water fleas (*Daphnia magna*) had an even higher 48-hr LC_{50} of 41,200 μg acenaphthene/L (U.S. EPA 1978b). The alga, *Selenastrum capricornutum*, showed comparable acute toxicity expressed as a 96-hr EC_{50} of 520 μg/L, based on cell numbers (U.S. EPA 1978b). The flow-through procedure employed in the present study probably accounts for these differences in toxicity (Nebeker and Puglisi 1974) although species differences are also undoubtedly important. The no-effect concentration determined in the present study (413 μg/L) is similar to the acutely toxic concentration (608 μg/L). This indicates that acenaphthene's effect on early-life-stage growth is a sensitive indicator of toxicity (Tables 1 and 2).

The 96-hr LC_{50} for isophorone (145 or 255 mg/L depending on age of fish used) was similar to results from static exposures: *S. capricornutum*—126 mg/L 96-h EC_{50} (U.S. EPA 1978b), bluegills—224 mg/L 96-hr LC_{50} (U.S. EPA 1978b), and 220 mg/L 96-hr LC_{50} (Buccafusco *et al.* 1981), and *D. magna*—117 mg/L 48-hr LC_{50} (U.S. EPA 1978b) and 120 mg/L 48-hr LC_{50} (Le Blanc 1980). The no-effect concentration for isophorone determined in the present study (14 mg/L) agrees well with the "no-discernible effect concentration" calculated for daphnia by Le Blanc (1980) of 15 mg/L.

The investigators also observed lethargic behavior and lack of alarm response in fish which were impacted by either chemical. This would indicate an increased susceptibility to predators and ecological death.

These flow-through toxicity tests with fathead minnows should add to the presently sparse data

base needed to set water quality criteria for acenaphthene and isophorone. Criteria based on direct toxic effects on aquatic organisms, as well as bioconcentration and human health considerations, are needed to protect the aquatic environment from deterioration by industrial chemicals. Although present environmental levels of these two chemicals are unknown, studies such as those reported herein provide biological effect levels which can be used to estimate potential damage to freshwater aquatic life.

Acknowledgments. The authors thank Alfred Sipols, Jean Waters, Claudia Wise, and Andrew Yartzoff for analytical support and James Andros for statistical assistance.

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Manuscript received March 22, 1982; accepted May 18, 1982.